# A Gene Drive for rabbit control

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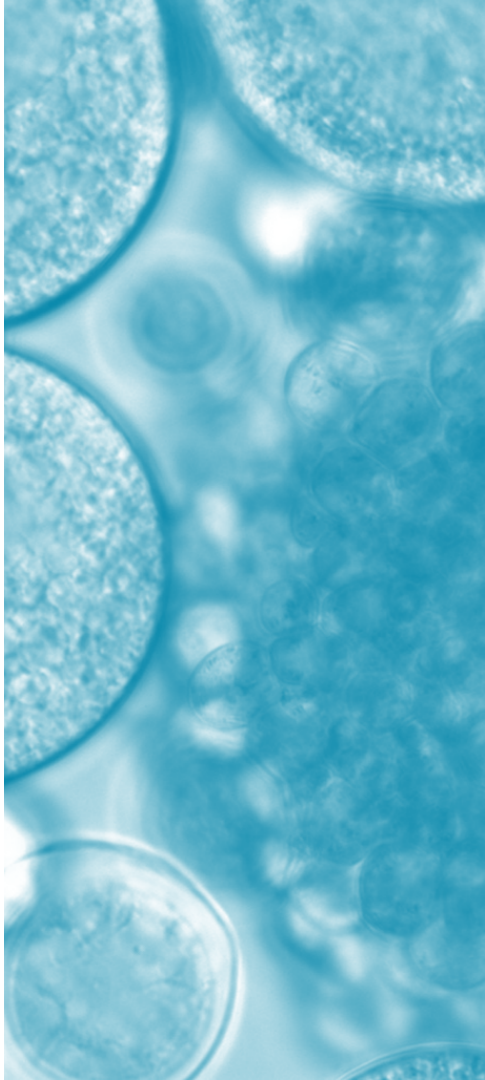
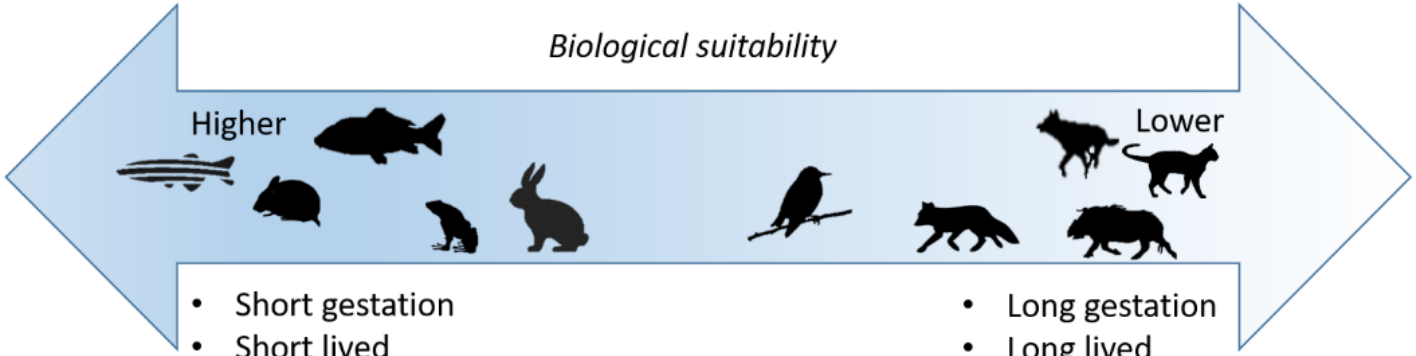
CSIRO



# Some species are more suitable than others…




- Short gestation
- Short lived
- High fecundity
- Small body size
- Captive breeding proven
- Short inter-generation period

- Long gestation
- Long lived
- Low fecundity
- Large body size
- Captive breeding challenging
- Long inter-generation period

# How do we move towards a gene drive for rabbit control?…


Mouse technical PoC (laboratory)

Mouse translation towards release

Release

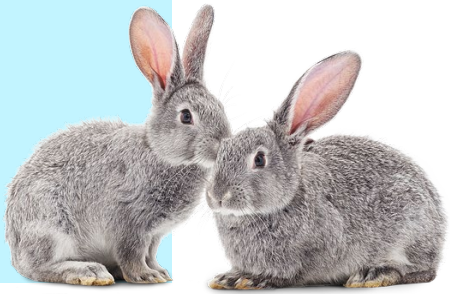
**Mouse application PoC!**

Transfer technology to other species (incl rabbits)

# Transfer technology to other species (rats, rabbits, cats, foxes, carp…)

Essential background data

- Population genomics (wild rabbits):
  - Genetic variation/gene flow/migration,etc
  - Scan for population specific gene drive targets (= Gene drive safety!)
- Biology:
  - Social structure
  - Behaviour
- Eco-genetic modelling



Essential technologies and infrastructure

- Reproductive technologies
- Efficient genetic engineering methods
- **Breeding colonies/ experimental facilities for genetically modified animals :**
  **=> domestic => wild**

Construct a working gene drive

- Gene editing methods
- Target gene validation
- ‘drive mechanism’

# Moving parts of a working gene drive

Improved gene editing methods
(cell culture/ animals)

Target gene validation in animals
(all male offspring, female infertile, etc)

Drive mechanism
(biasing inheritance)

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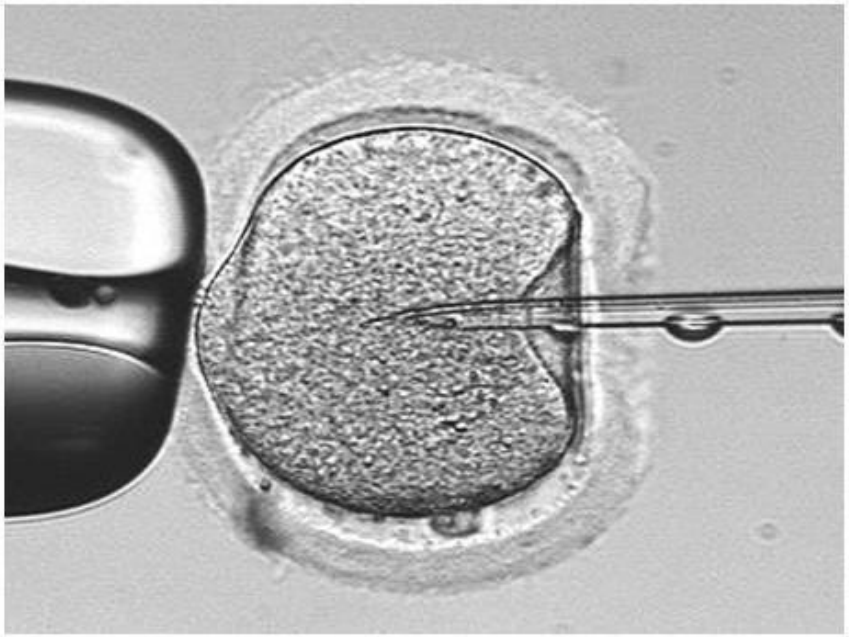
(biasing inheritance)

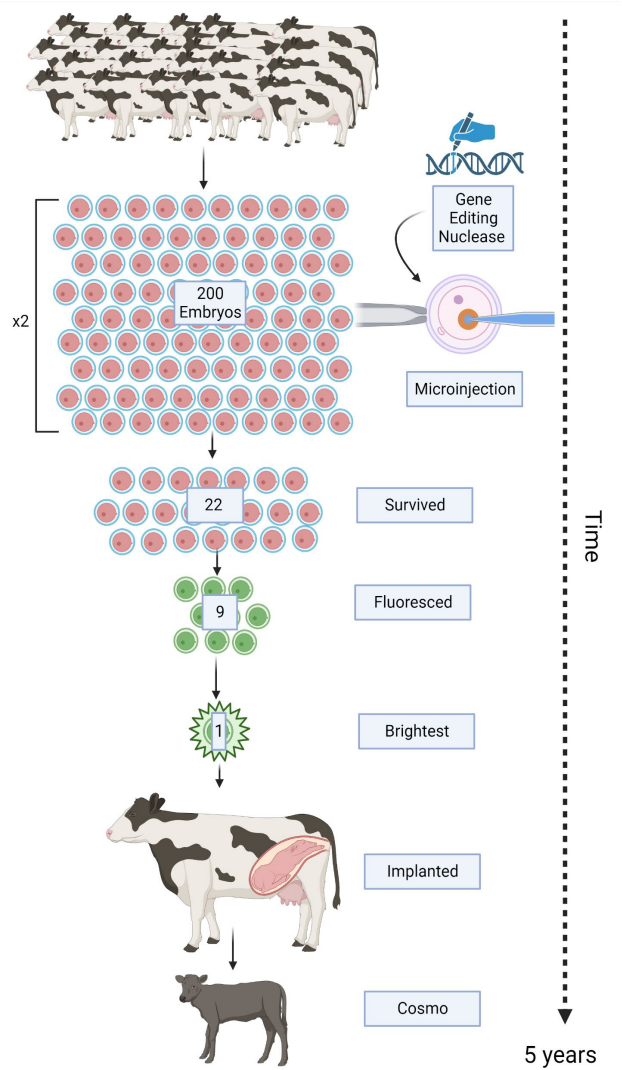
UoA: mice, rats
CSIRO: rabbits

UoM: zebra fish, carp, toads, cats, foxes, rabbits
MU: zebra fish, cane toads
UTas: Gambusia

# Making Genetically Modified Mammals



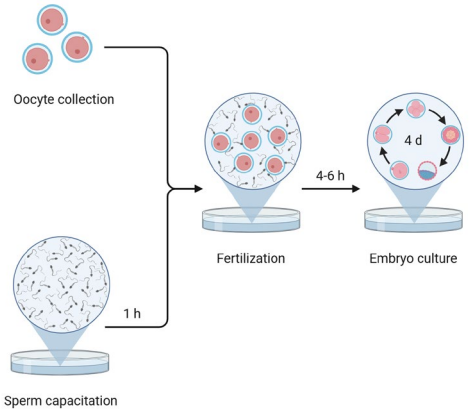
- The most common techniques globally to produce genetically modified mammals are microinjection and zygote electroporation.
- These protocols are well established in mice, but far less so in other species.
- Both techniques are highly inefficient in most mammalian species, often taking years to produce a single modified animal.




# Cosmo the Cow

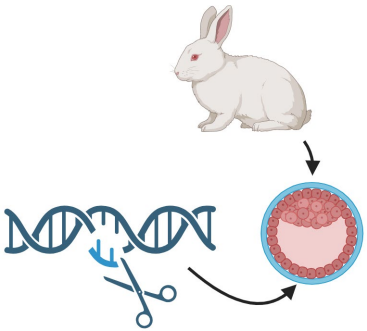
# Areas of Focus to Improve the Feasibility of Genetic Biocontrol

## Area 1




Adapting protocols for in vitro fertilisation (IVF) of rabbit embryos

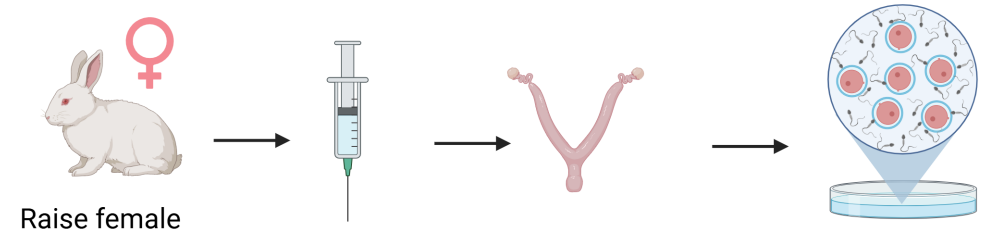
## Area 2



Increasing the efficiency of genome engineering strategies in rabbit embryos

# Area 1 - Rabbit IVF

- Rabbits are induced ovulators, meaning ovulation is triggered by copulation or hormone injections
- IVF in literature exclusively uses superovulated laboratory rabbits (+6 months old) which must be humanely killed to harvest the oocytes
- Our small PC2 colony of laboratory rabbits is essential for many components of our work including semen collection, viral biocontrol work and embryo implantation.
- However at our current capacity, our facility cannot support oocyte retrieval at the level that would be required for us to perform all our necessary experiments.

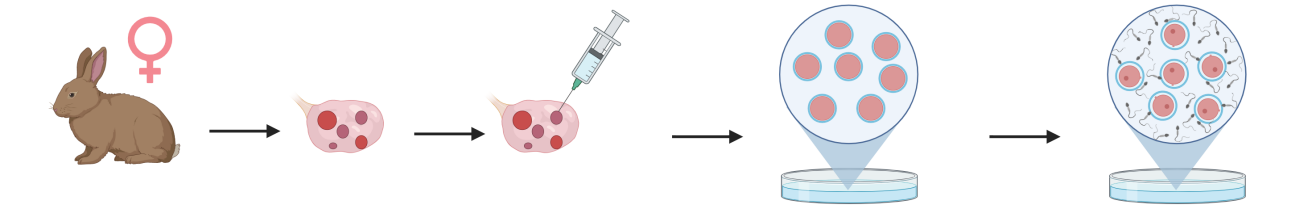


# ACT Parks Rabbit Control Program

- ACT Parks conducts weekly shooting operations around the Canberra area to control rabbits
- ACT Parks have been generous in allowing us to attend these shoots
- Mature females must be processed quickly to obtain viable oocytes = mobile field lab!

# IVF Using Wild Rabbits

- Wild rabbits are not superovulated, meaning the oocytes we would collect are mostly immature with reduced fertilisation capacity
- A common problem also faced by researchers performing IVF in cattle who collect oocytes directly from ovaries sourced from abattoirs
- In collaboration with other scientists at CSIRO who do bovine IVF we achieved **ex vivo** maturation of oocytes



Wild shot mature female rabbits

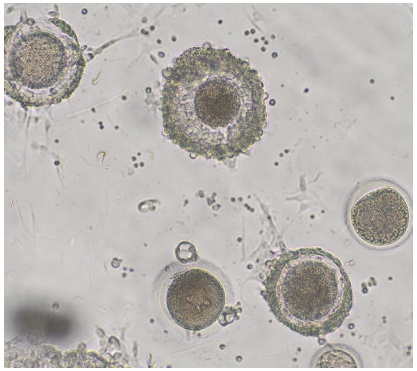
Collect ovaries

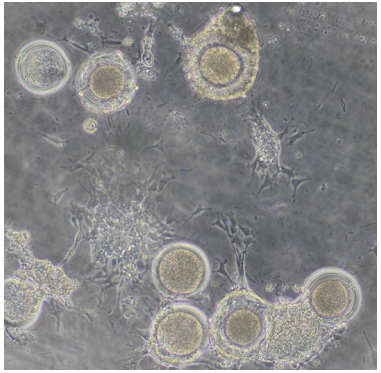
Use a needle to aspirate follicles

Collect oocytes and place into a maturation media containing FSH hormone for 18 hours

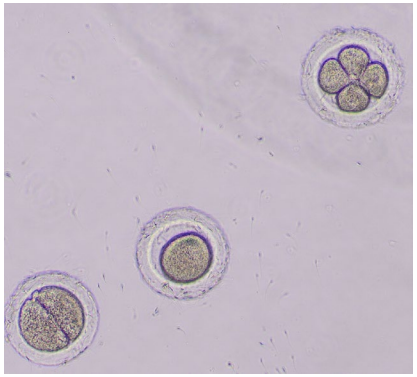
Place oocytes into fertilisation media and add sperm to allow fertilisation

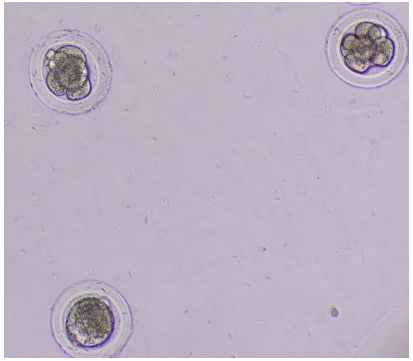
Day 0
Matured Eggs



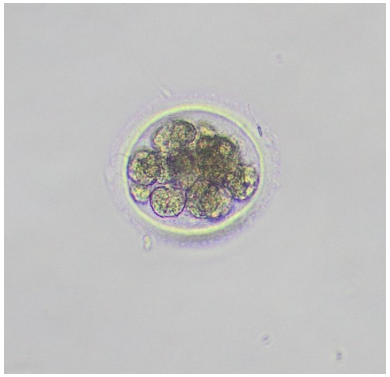


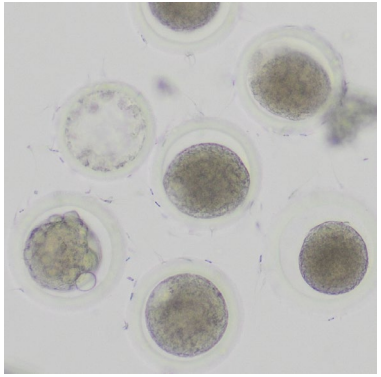
Day 1
Early Embryos



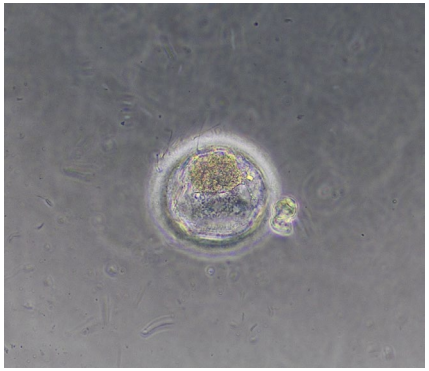


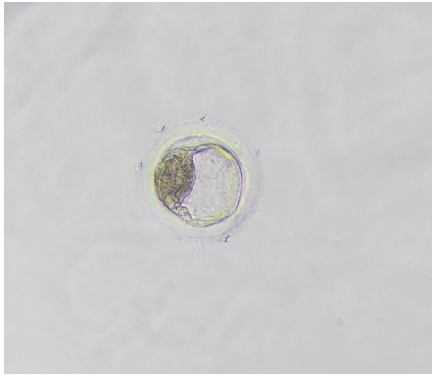
Day 3
Morulas





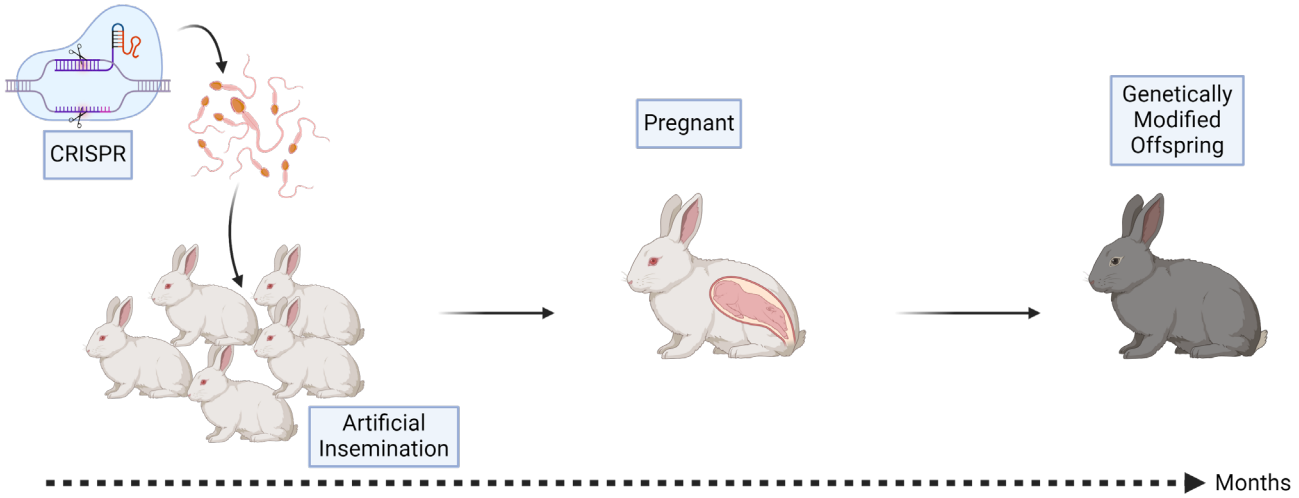
Day 7
Blastocysts





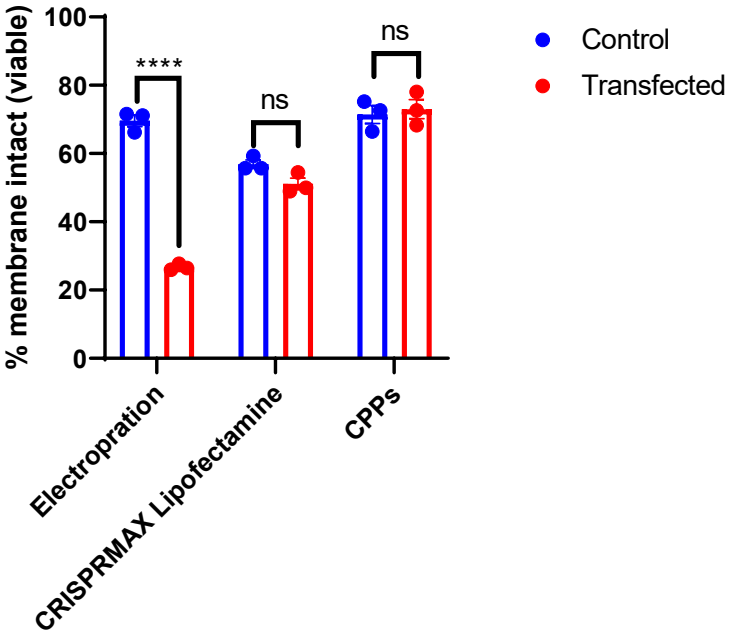
# Area 2 - Sperm Transfection Assisted Gene Editing (STAGE)

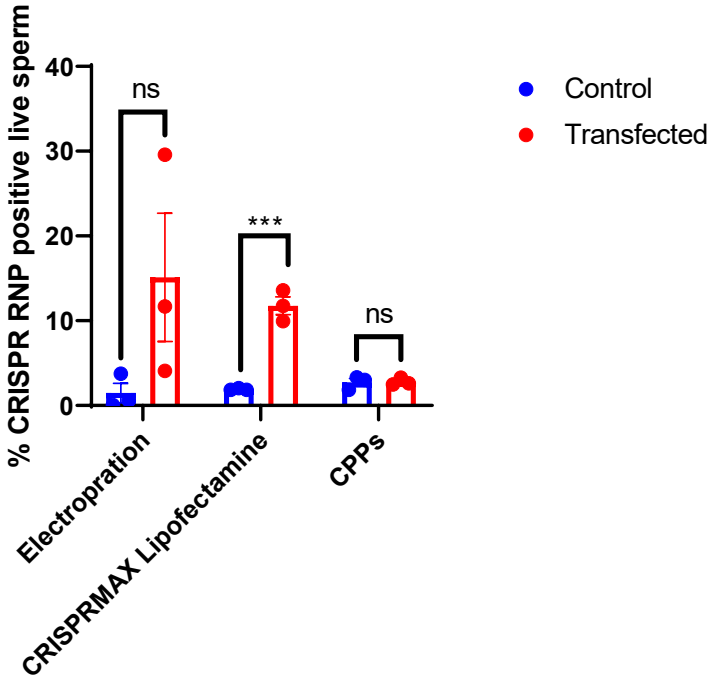
- STAGE is a CSIRO developed technology, first demonstrated in chickens by the H&B genome editing team in Geelong.
- We are aiming to apply STAGE to rabbits for the first time
- It relies of the genome editing material (CRISPR) being packaged inside the sperm and delivered to the embryo



# Sperm Transfection Optimisation

- We found a method that resulted in a significant increase in CRISPR RNP uptake in sperm, with the sperm still viable



# Conclusion

- We have successfully adapted a rabbit protocol using ex vivo matured oocytes to enable a continual source of rabbit embryos to test genome engineering strategies in vitro
- We have been able to transfect rabbit sperm with CRISPR RNP while maintaining the viability of the sperm

# Next Steps

- We have already begun to trial genome engineering strategies on embryos. We are in the process of screening embryos to know whether these efforts have been successful.
- Next steps will be to continue to optimise genome engineering strategies in vitro and develop the technique of embryo implantation to put our genetically modified embryos into donor females.

# Acknowledgements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